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(71) Applicant: PURDUE RESEARCH FOUNDATION [US/US]; 1291 Cumberland Avenue, West Lafayette, IN 47906 (US).

(72) Inventor: KINCH, Michael, Scott; 2320 Flowering Crab Drive, Lafayette, IN 47905 (US).

(74) Agent: SANDBERG, Victoria, A.; Mueting, Raasch & Gebhardt, P.A., P.O. Box 581415, Minneapolis, MN 55458-1415 (US).

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(54) Title: TREATMENT OF METASTATIC DISEASE

(57) Abstract: The present invention is directed to compounds and methods for the treatment of metastatic disease. The compounds of this invention have specificity for EphA2, an epithelial cell tyrosine kinase that is overexpressed in metastatic tumor cells. The compounds used in accordance with this invention may be provided in a pharmaceutical composition for treatment of metastatic disease.

AMENDED CLAIMS

[received by the International Bureau on 16 March 2001 (16.03.01);
original claims 1, 16 and 19 amended; claims 20-22 renumbered 21-23;
new claims 20 and 24-57 added; remaining claims unchanged (7 pages)]

1. A pharmaceutical composition for treatment of a mammalian metastatic tumor including a population of cells which overexpress tyrosine kinase EphA2 having an amino acid sequence defining at least one extracellular epitope, said composition comprising a compound that specifically interacts with the extracellular epitope of EphA2 to reduce metastatic proliferation of said tumor, and a pharmaceutically acceptable carrier.

2. The composition of claim 1 wherein the compound is an antibody.

3. The composition of claim 2 wherein the antibody is a monoclonal antibody.

4. The composition of claim 3 wherein the antibody is the monoclonal antibody B2D6.

5. The composition of claim 2 wherein the antibody is conjugated to a cytotoxic agent.

6. The use of a compound for the manufacture of a medicament for the treatment of a metastatic tumor comprising a population of cells that overexpress EphA2, said compound having specificity for EphA2.

7. The use of claim 6 wherein the compound is an antibody having specificity for an extracellular epitope of EphA2.

8. The use of claim 7 wherein the antibody is a monoclonal antibody.

9. The use of claim 8 wherein the antibody is produced from a hybridoma cell line identified as B2D6.

10. The use of claim 7 wherein the antibody selectively binds to metastatic cells.

11. The use of claim 10 wherein the antibody reduces proliferation of metastatic tumor cells.

12. The use of claim 6 wherein the population of cells forms at least a portion of a cancer tumor selected from the group consisting of breast, prostate, lung, and colon cancer tumors.

13. The use of claim 6 wherein the compound is an ephrin that affects phosphorylation of EphA2.

14. A method for detecting the presence of metastatic cells in a cell population comprising

providing an antibody specific to an extracellular epitope of EphA2,
binding a detectable label to the antibody,
providing a cell sample,
incubating the cell sample with the labeled antibody,
removing unbound antibody from the cell sample, and
detecting the presence of the label.

15. A method of producing an antibody which inhibits the proliferation of metastatic tumor cells comprising

injecting tyrosine phosphorylated proteins into lymph nodes of a mammal,
harvesting lymph node cells from the mammal,
fusing the lymph node cells with myeloma cells to form hybridomas,
selecting at least one hybridoma producing an antibody which specifically binds to an extracellular epitope of EphA2, and
isolating said antibody.

16. A method of producing an antibody which inhibits the proliferation of metastatic tumor cells comprising injecting tyrosine phosphorylated proteins proximal to lymph nodes of a mammal,

harvesting lymph node cells from the mammal,

fusing the lymph node cells with myeloma cells to form hybridomas,

selecting at least one hybridoma producing an antibody which specifically binds to an extracellular epitope of EphA2, and

isolating said antibody.

17. The method of claim 15 wherein the tyrosine phosphorylated proteins comprise EphA2.

18. The antibody produced by the method of claim 15.

19. A pharmaceutical composition for treatment of a mammalian metastatic tumor, said composition comprising a compound that interferes with EphA2 function to reduce metastatic proliferation of said tumor, and a pharmaceutically acceptable carrier.

20. The pharmaceutical composition of claim 19 wherein the compound is a peptide.

21. The pharmaceutical composition of claim 19 wherein the compound is an agonist.

22. The pharmaceutical composition of claim 20 wherein the compound comprises a peptide sequence defining an extracellular domain of EphrinA1.

23. The pharmaceutical composition of claim 21 wherein the peptide sequence is linked to a second peptide sequence defining immunoglobulin heavy chain.

24. A pharmaceutical composition for treatment of a mammalian metastatic tumor including a population of cells which overexpress tyrosine kinase EphA2 having an amino acid sequence defining at least one extracellular epitope, said composition comprising a compound that specifically interacts with the extracellular epitope of EphA2 to reduce metastatic invasion or proliferation of said tumor, and a pharmaceutically acceptable carrier.

25. The composition of claim 24 wherein the compound comprises an antibody.

26. The composition of claim 25 wherein the antibody is produced by hybridoma cell line B2D6.

27. The composition of claim 25 wherein the antibody is conjugated to a cytotoxic agent.

28. The compound of claim 27 wherein the cytotoxic agent is selected from the group consisting of a bacterial toxin, ricinA-chain, daunorubicin, methotrexate, a ribosome inhibitor, and a radioisotope.

29. The compound of claim 28 wherein the cytotoxic agent is a radioisotope selected from the group consisting of an alpha emitter, a beta emitter, and an Auger electron emitter.

30. A pharmaceutical composition for treatment of a mammalian metastatic cancer, the composition comprising a compound having at least one biological activity selected from the group consisting of an ability to specifically bind to EphA2, an ability to alter the expression of EphA2, and an ability to stimulate EphA2, said biological activity associated with a reduction in invasiveness, metastatic proliferation, or both of the metastatic cancer; and a pharmaceutically acceptable carrier.

31. The pharmaceutical composition of claim 30 wherein the compound comprises an antibody or an ephrin.

32. An antibody which specifically binds to an extracellular epitope of EphA2.

33. The antibody of claim 32 which is a monoclonal antibody.

34. The antibody of claim 32 bound to a detectable label.

35. The antibody of claim 32 that is produced by hybridoma cell line B2D6.

36. The antibody of claim 32 which is a humanized antibody.

37. An isolated antibody which specifically binds to an extracellular epitope of EphA2.

38. A compound comprising an antibody that specifically binds to an epitope of extracellular EphA2; and a cytotoxic agent.

39. The compound of claim 38 wherein the antibody is produced by hybridoma cell line B2D6.

40. The compound of claim 38 wherein the cytotoxic agent is selected from the group consisting of a bacterial toxin, ricinA-chain, daunorubicin, methotrexate, a ribosome inhibitor, and a radioisotope.

41. The compound of claim 38 wherein the cytotoxic agent is a radioisotope selected from the group consisting of an alpha emitter, a beta emitter, and an Auger electron emitter.

42. Hybridoma cell line B2D6.

43. Isolated hybridoma cell line B2D6.

44. A monoclonal antibody produced by hybridoma cell line B2D6.

45. An isolated monoclonal antibody produced by hybridoma cell line B2D6.

46. A method of treatment of a patient having a metastatic or potentially metastatic cancer comprising a population of cells that overexpress EphA2, said method comprising administering a therapeutically effective amount of a compound that targets EphA2.

47. The method of claim 46 wherein the compound comprises an antibody having specificity for EphA2.

48. The method of claim 47 wherein the antibody has specificity for an extracellular epitope of EphA2.

49. The method of claim 47 wherein the antibody selectively binds to metastatic cells.

50. The method of claim 47 wherein the antibody is produced from hybridoma cell line B2D6.

51. The method of claim 46 wherein administration of the compound prevents or slows metastasis of potentially metastatic cancer cells.

52. The method of claim 46 wherein administration of the compound reduces or prevents proliferation of metastatic cancer cells.

53. The method of claim 46 wherein administration of the compound reduces or prevents tissue invasion by metastatic cancer cells.

54. The method of claim 46 wherein the population of cells comprises cells selected from the group consisting of breast cancer cells, prostate cancer cells, lung cancer cells and colon cancer cells.

55. The method of claim 46 wherein the compound comprises an antisense oligonucleotide that affects EphA2 expression.

56. The method of claim 46 wherein the cells that overexpress EphA2 are epithelial cells.

57. A method for identifying an EphA2 antibody that is selective for EphA2 in metastatic cancer cells, the method comprising:

comparing the binding of an EphA2 antibody as a function of cell density in noncancerous cells to the binding of the EphA2 antibody as a function of cell density in analogous metastatic cancer cells;

wherein an observed decrease in antibody binding to noncancerous cells as cell density increases, coupled with an observed constant level of antibody binding to the metastatic cancer cells as cell density increases, is indicative of an EphA2 antibody selective for EphA2 in metastatic cancer cells.

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8. The use of claim 7 wherein the antibody is a monoclonal antibody.

9. The use of claim 8 wherein the antibody is produced from a hybridoma cell line identified as B2D6.

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